

## Chapter XIV

### EVALUATION OF HEPATIC STATUS

#### 1. Introduction

A very broad spectrum of hepatic phenomena has been reported in association with acute, subacute and chronic administration of TCDD to animals. Significant response differences between species occur, however. Serum enzyme changes (SGOT, SGPT, GGPT, LDH) have not been prominent, although SGPT levels were elevated in at least 1 study (Schantz et al, 1979). Elevated alkaline phosphatase levels have been observed with increased direct bilirubin levels (Kociba et al, 1976). Decreased serum cholesterol levels have also been noted after sublethal exposures (Schantz et al, 1979). TCDD interferes with hemoglobin metabolism affecting delta-aminolevulinic acid synthetase activity (Goldstein et al, 1973) and possibly other enzyme activities, providing, at sufficient doses, signs and symptoms of porphyria.

Motivated by the literature reports of hepatotoxicity, signs and symptoms of hepatic dysfunction were sought in the participants in this study. In this chapter, enzyme levels, bilirubin levels and lipid values are presented, along with determinations reflecting porphyrin metabolism. Clinical history data are also analyzed, along with hepatomegaly determined at physical examination.

#### 2. Biochemical Determinations

##### a. Analyses Overview

In this section 9 biochemical determinations are studied: SGOT, SGPT, GGPT, alkaline phosphatase (Alk. Phos.), total bilirubin (T. Bil), direct bilirubin (D. Bil), lactic dehydrogenase (LDH), cholesterol (Chol) and triglycerides (Trig). These 9 variables are listed in Table XIV-1, along with the normal-abnormal ranges used in the reported statistical analyses. These ranges were adapted from Kelsey-Seybold laboratory normal ranges.

In the analyses of these 9 variables, adjustments were made for 4 covariates: current alcohol ingestion (ALC), days of exposure to industrial chemicals (IC), days of exposure to degreasing chemicals (DC), and presence or absence of antibody to hepatitis B surface antigen (anti-HB<sub>s</sub>Ag). The current alcohol use covariate was taken from the personal medical history administered at the time of the physical examination and is in units of average drinks per day (see Appendix VI, page 2). Current alcohol ingestion was selected as an adjusting variable over the drink years measure developed from the questionnaire, since preliminary testing indicated it correlated better with hepatic endpoints. The industrial chemical and degreasing chemical exposures were derived from the in-home questionnaire (total unprotected exposure).

The data analyzed were from the entire Ranch Hand cohort compliant to the physical examination (N = 1045) and the original comparisons compliant to the physical examination (N = 773). Ten Ranch Handers and 2 comparisons were removed from the analysis because of body temperature of 100°F or more, and the effect of fever on hepatic variables. Individuals whose blood contained hepatitis B surface antigen (HBsAg) were also removed from the analysis (8 Ranch Handers and 7 comparisons).

#### b. Group Analyses

Three sets of analyses were run:

(1) Continuous-continuous analyses (CC): In these evaluations both the dependent variables and adjusting covariates, except anti-HBsAg which is dichotomous, were used as continuous variables in an analysis of covariance.

(2) Continuous-discrete analyses (CD): In these analyses all 4 covariates were used as dichotomous variables while the dependent variables were maintained as continuous.

(3) Discrete-discrete analyses (DD): All variables were analyzed in dichotomous form using the log-linear model for discrete data.

In all 3 analysis settings, group-by-covariate interactions were examined. In addition, the continuous-continuous and continuous-discrete analyses models were fit without interaction terms to provide discussion of appropriate tests when dependent variable relationships with the covariates are the same in both groups. In the continuous-continuous and continuous-discrete analyses the dependent variable was normalized by using a logarithmic (base 10) transformation.

Table XIV-1

#### NORMAL - ABNORMAL LEVELS OF NINE BIOCHEMICAL DETERMINATIONS REFLECTING HEPATIC FUNCTION

<u>Determination</u>	<u>Normal</u>	<u>Abnormal</u>
1. SGOT	≤ 41	> 41
2. SGPT	≤ 45	> 45
3. GGPT	≤ 85	> 85
4. Alkaline Phosphatase	≤ 9.7	> 9.7
5. Total Bilirubin	≤ 1.2	> 1.2
6. Direct Bilirubin	≤ 0.36	> 0.36
7. Lactic Dehydrogenase	≤ 200	> 200
8. Cholesterol	≤ 240	> 240
9. Triglycerides	≤ 150	> 150

Table XIV-2 provides unadjusted means, adjusted means, and percent abnormality by groups for the 9 hepatic-related variables. A summary of the 3 classes of analyses is provided in Table XIV-3. The results in this table provide P values for Ranch Hand-comparison group differences.

Table XIV-2

UNADJUSTED MEANS, ADJUSTED MEANS AND PERCENT ABNORMALITY FOR  
NINE LIVER-RELATED VARIABLES

<u>Variable</u>	<u>Group</u>	<u>Unadjusted Means</u>	<u>Adjusted Means</u>	<u>Percent Abnormality</u>
SGOT	RH	33.0	33.0	13.9
	COM*	33.1	33.1	14.8
SGPT	RH	20.3	20.3	7.8
	COM	20.5	20.5	8.6
GGPT	RH	40.2	40.1	10.8
	COM	39.3	39.3	10.3
Alk. Phos.	RH	7.68	7.69	17.3
	COM	7.53	7.52	16.9
T. Billi	RH	0.57	0.57	1.8
	COM	0.58	0.58	2.0
D. Billi	RH	0.23	0.23	29.0
	COM	0.24	0.24	29.7
LDH	RH	142.1	142.1	1.7
	COM	141.7	141.7	2.1
CHOL	RH	212.2	212.2	26.0
	COM	216.6	216.6	27.7
TRIG	RH	121.8	121.9	34.7
	COM	124.3	124.1	36.1

\*COM denotes original fully compliant comparisons.

Table XIV-3

SUMMARY OF RESULTS  
UNMATCHED ANALYSES OF NINE BIOCHEMICAL VARIABLES REFLECTING LIVER FUNCTION

P Values for Models with interaction										P Values for models without interaction					
VAR	ANAL	Gp	ALC	IC	DC	anti HB <sub>s</sub> Ag	Gp X ALC	Gp X IC	Gp X DC	Gp X anti HB <sub>s</sub> Ag	Gp	ALC	IC	DC	anti HB <sub>s</sub> Ag
SGOT	CC	.127	<.001	—*	—	—	.032	—	—	—	.805	<.001	—	—	—
	CD	.278	<.001	—	—	—	—	—	—	—	.867	<.001	—	—	—
	DD	.578	<.001	—	—	—	—	—	—	—	—	—	—	—	—
SGPT	CC	.736	<.001	—	—	—	—	—	—	—	.663	<.001	—	—	—
	CD	.309	.005	—	—	—	—	—	—	—	.662	.003	—	—	—
	DD	.592	—	—	—	—	—	.052	—	—	—	—	—	—	—
GGPT	CC	.731	<.001	—	—	—	—	—	—	—	.483	<.001	—	—	—
	CD	.050	<.001	—	—	.066	—	—	—	—	.421	<.001	—	—	.078
	DD	.782	<.001	—	—	—	—	—	—	—	—	—	—	—	—
ALK PHOS	CC	.405	—	—	—	.009	—	—	—	—	.140	—	.071	—	.009
	CD	.142	.001	—	—	.010	—	—	—	—	.115	.001	.066	—	.011
	DD	.734	—	—	—	—	—	—	—	—	—	—	—	—	—
TOT BILI	CC	.113	.014	.036	.001	.100	—	—	—	—	.423	.009	.011	<.001	.095
	CD	.606	—	—	—	—	—	—	—	—	.400	—	—	—	.099
	DD	.800	—	—	—	.027	—	—	—	—	—	—	—	—	—
DIR BILI	CC	.494	.004	—	.032	—	—	—	—	—	.770	.003	—	.016	—
	CD	.371	.091	—	—	—	.069	—	—	—	.755	—	—	—	—
	DD	.869	—	—	—	—	—	—	—	—	—	—	—	—	—
LDH	CC	.063	.090	—	—	—	.011	—	.037	—	.836	.025	—	.023	—
	CD	.024	—	—	—	—	—	—	—	—	.711	—	—	—	—
	DD	.526	—	—	—	—	.086	—	—	—	—	—	—	—	—
CHOL	CC	.062	<.001	.079	—	—	—	—	—	—	.022	<.001	.061	—	—
	CD	.216	.014	—	—	—	—	—	—	—	.031	.020	—	—	—
	DD	.466	.053	—	—	—	—	—	—	—	—	—	—	—	—
TRIG	CC	.911	—	—	—	—	—	—	—	—	.601	—	—	—	—
	CD	.284	—	—	—	—	—	—	—	—	.616	—	—	—	—
	DD	.589	—	—	—	—	—	—	—	—	—	—	—	—	—

\* — denotes  $P > 0.050$  for main effects,  $P > 0.100$  for interaction effects

In Tables XIV-2 and XIV-3, there is a very slight indication of overall group differences in the GGPT with the Ranch Hand mean greater than the comparison mean and a P value of 0.050 in the CD analysis with interaction terms. However, when interaction terms are not considered,  $P = 0.421$ . This may indicate some interaction effects even though they were not detected as statistically significant. Additionally, no difference is detected in the CC or DD

analyses. A stronger indication of overall group difference is seen with LDH; however, it is interesting to note that while the Ranch Hand mean LDH is greater than the comparison mean, the Ranch Hand percent abnormal LDH is less than that of the comparison group. The Ranch Hand cholesterol mean is lower than that of the comparison group and the result appears unlikely to have occurred by chance (P value of 0.062 in the full model CC analysis; P values of 0.022 and 0.031 in the CC and CD analyses respectively not using interaction terms). These group differences in GGPT, LDH and CHOL are all small.

Further group specific differences are noted in interaction effects with covariables. Ranch Hand SGOT levels are correlated more highly with alcohol ingestion than are comparison SGOT levels. The Ranch Hand SGOT - alcohol regression slope is 0.0178 logarithmic units per drink per day, while the comparison SGOT - alcohol slope is 0.0113 logarithmic units per drink per day. This difference in slopes is statistically significant with  $P = 0.032$ , and could represent differing hepatic sensitivities to alcohol.

A borderline group by industrial chemical exposure is noted in the DD analysis of SGPT levels. This interaction is shown in Table XIV-4.

Table XIV-4

INDUSTRIAL CHEMICAL EXPOSURE AND % ABNORMAL SGPT IN  
RANCH HAND AND COMPARISON GROUPS

	<u>Ranch Hand</u>	<u>Comparison</u>
Exposure	8.84% (38 of 430)	6.71% (23 of 343)
No Exposure	7.19% (42 of 584)	10.1% (42 of 416)

Ranch Hand personnel exposed to industrial chemicals have a higher proportion of abnormal SGPT values than do Ranch Hand personnel who are not exposed to industrial chemicals. The situation is reversed in the comparison group. The relative risk for abnormal SGOT in the Ranch Hand group associated with industrial chemical exposure is 1.23, while the comparison relative risk is 0.66, and this difference carries a P value of 0.052.

Two group-by-covariate interactions are noted in the LDH data. In the comparison group neither alcohol ingestion nor exposure to degreasing chemicals was associated with change in LDH levels, while in the Ranch Hand group, increased levels were noted to occur in association with both exposures. Specifically, in the comparison group the LDH-alcohol slope is -0.0008 logarithmic units per drink per day which is not statistically significantly different from

zero ( $P = 0.577$ ). Also, the comparison LDH-degreasing chemical slope is  $-0.08 \times 10^{-5}$  units per exposure day ( $P = 0.735$  against the null hypothesis of zero slope). On the other hand, the Ranch Hand LDH-alcohol slope is 0.0041 units per drink per day ( $P < 0.001$  against hypothesis of zero slope) and the LDH-degreasing slope is  $0.51 \times 10^{-5}$  units per exposure day ( $P = 0.003$  against zero slope hypothesis).

### c. Exposure Analyses

Analyses within the Ranch Hand cohort are presented contrasting the hepatic clinical variables against the herbicide exposure index. For this exposure index work, separate analyses were run for each of 3 occupational groups: officers, enlisted flying and enlisted ground. The 9 hepatic variables were analyzed as continuous dependent variables after logarithmic transformation. As with the Ranch Hand-comparison group analyses, alcohol use, industrial chemical exposure, degreasing chemical exposure and antibody to Hepatitis B surface antigen were used as adjusting covariates, and individuals with body temperature greater than or equal to 100°F were omitted from the analysis as were individuals with hepatitis B surface antigen. For this exposure index effort, alcohol use, industrial chemical exposure and degreasing chemical exposure were used as continuous variables.

Table XIV-5 is a display of exposure means adjusting for covariates without invoking interaction. Table XIV-6 provides a summary of P values for the testing. Analyses of covariance or generalized linear models with and without interaction were employed.

An overall or main exposure effect on GGPT levels is indicated among officers and enlisted ground personnel. However, clear-cut dose-response patterns are not noted, rather, in the officer cohort the medium exposure subgroup has the highest mean GGPT while in the enlisted ground cohort the subgroup with low exposure has the highest GGPT.

Six exposure group-by-covariate interactions were found at  $P \leq 0.050$ . These interactions are written out in Table XIV-7. In this table, the slope of the dependent variable with respect to the covariate of interest is provided for each of the 3 exposure levels.

An exposure-by-degreasing chemical interaction was noted in SGOT in officers. Low herbicide exposure is associated with a possible depression of SGOT levels with increasing degreasing chemical exposure, while individuals in the high herbicide exposure group show increasing SGOT levels with increasing degreasing chemical exposure.

Table XIV-5

ADJUSTED BIOCHEMICAL MEANS BY EXPOSURE AND OCCUPATIONAL  
CATEGORY, WITH TYPICAL SAMPLE SIZES

<u>Variable</u>	<u>Occupational Category</u>	<u>Low Exposure</u>	<u>Medium Exposure</u>	<u>High Exposure</u>
SGOT	Officer	33.3	32.2	33.0
	Enl. F.	31.8	33.5	31.7
	Enl. G.	33.6	32.7	34.1
SGPT	Officer	20.2	19.9	19.4
	Enl. F.	18.5	20.8	18.4
	Enl. G.	21.3	21.1	20.6
GGPT	Officer	37.1	39.5	37.5
	Enl. F.	41.4	45.9	37.8
	Enl. G.	43.0	40.2	40.5
Alk. Phos.	Officer	6.91	7.24	7.47
	Enl. F.	8.13	7.88	7.98
	Enl. G.	7.93	7.85	8.04
T. Billi.	Officer	0.56	0.55	0.57
	Enl. F.	0.53	0.56	0.54
	Enl. G.	0.58	0.58	0.60
D. Billi.	Officer	0.22	0.23	0.23
	Enl. F.	0.18	0.23	0.21
	Enl. G.	0.25	0.24	0.26
LDH	Officer	141.3	139.4	139.3
	Enl. F.	143.1	141.0	149.3
	Enl. G.	142.9	140.8	144.9
Chol.	Officer	214.6	213.0	209.4
	Enl. F.	214.0	212.6	222.5
	Enl. G.	208.7	210.4	211.4
Trig.	Officer	111.9	127.4	129.0
	Enl. F.	129.8	126.4	128.4
	Enl. G.	118.6	114.5	121.1
Typical Sample Sizes	Officer	107	122	120
	Enl. F.	58	58	63
	Enl. G.	143	170	146

Table XIV-6

SUMMARY OF P VALUES FOR EXPOSURE INDEX ANALYSIS  
OF NINE HEPATIC VARIABLES

P Values for Models with Interaction										P Values for Models With No Interaction					
VAR	OCC CAT	EXP CAT	ALC	IC	DC	aHb	EXP X ALC	EXP X IC	EXP X DC	EXP X anti HBsAg	Exp Cat	ALC	IC	DC	anti HBsAg
SGOT	OFF	.563	<.001	—*	—	—	—	—	.009	—	.512	<.001	—	.047	—
	ENL.F.	.885	<.001	—	—	.037	—	—	—	—	.538	<.001	—	—	.035
	ENL.G.	.698	<.001	—	—	—	—	—	—	—	.409	<.001	—	—	—
SGPT	OFF	.463	<.001	—	—	—	—	.081	—	—	.812	<.001	—	—	—
	ENL.F.	.909	—	—	—	—	—	—	—	—	.411	—	—	—	—
	ENL.G.	.467	—	—	—	—	—	—	—	—	.862	—	—	—	—
GGPT	OFF	.052	<.001	—	—	—	.089	—	—	—	.696	<.001	—	.040	—
	ENL.F.	.427	<.001	—	—	—	.049	—	—	—	.224	<.001	—	—	—
	ENL.G.	.093	<.001	—	.010	—	—	—	—	—	.574	<.001	—	.020	—
ALK PHOS	OFF	.192	—	—	—	—	<.001	—	—	—	.280	—	—	—	—
	ENL.F.	.685	—	—	—	—	—	—	—	—	.855	—	—	—	—
	ENL.G.	.629	—	—	—	—	—	—	—	—	.710	—	—	—	—
TOT BILI	OFF	.643	—	—	—	—	—	—	—	—	.885	—	—	—	—
	ENL.F.	.449	.029	—	—	—	—	—	—	.086	.560	.011	—	—	—
	ENL.G.	.606	—	—	.010	—	—	—	—	—	.642	—	.023	.008	—
DIR BILI	OFF	.992	—	—	—	—	—	—	—	—	.856	—	—	—	—
	ENL.F.	.399	—	—	—	—	—	—	.060	.006	.310	—	—	—	—
	ENL.G.	.823	—	—	—	—	—	—	—	—	.697	—	—	—	—
LDH	OFF	.516	—	—	—	—	—	—	—	—	.758	—	—	—	—
	ENL.F.	.656	.018	—	—	—	—	—	—	—	.174	.019	—	—	—
	ENL.G.	.300	—	.050	—	—	—	—	—	.049	.360	.034	.036	—	—
CHOL	OFF	.290	—	—	—	—	—	—	—	—	.602	—	—	—	—
	ENL.F.	.310	.031	—	—	—	—	—	—	—	.343	.037	—	—	—
	ENL.G.	.096	—	—	—	—	.026	—	.058	—	.841	—	—	—	—
TRIG	OFF	.394	—	—	—	—	—	—	—	—	.244	—	—	—	—
	ENL.F.	.468	.045	.044	—	—	—	—	—	—	.980	—	—	—	—
	ENL.G.	.890	—	—	—	—	—	—	—	—	.768	—	—	—	—

\* - indicates P &gt; 0.050 for main effects P &gt; 0.100 for interactions.



Table XIV-7  
EXPOSURE - COVARIATE INTERACTION EFFECTS FOR NINE  
HEPATIC VARIABLES

Var	Occ Cat	Interact	Level of Interact	Exposure Level	Slope	P Value on Test of Slope Against Null Hypothesis of Zero Slope
SGOT	Officers	Exp x DC	.009	Low	$-.201 \times 10^{-4}$ units/day	.286
				Med	$.021 \times 10^{-4}$ units/day	.924
				High	$.674 \times 10^{-4}$ units/day	.002
GGPT	Enlisted Flying	Exp x ALC	.049	Low	.0828 units/drck/day	<.001
				Med	.0561 units/drck/day	.002
				High	.0288 units/drck/day	.037
ALK PHOS	Officers	Exp x ALC	<.001	Low	-.0442 units/drck/day	<.001
				Med	.0131 units/drck/day	.254
				High	-.0015 units/drck/day	.864
DIR BILI	Enlisted Flying	Exp x Anti Hb <sub>s</sub> Ag	.006	Low	.3713 mgm/dl	.013
				Med	-.2246 mgm/dl	.071
				High	.1752 mgm/ml	.134
LDH	Enlisted Ground	Exp x Anti Hb <sub>s</sub> Ag	.049	Low	.0329 units	.159
				Med	-.0407 units	.085
				High	-.0330 units	.128
CHOL	Enlisted Ground	Exp x ALC	.026	Low	.0039 mgm/dl/drck/day	.284
				Med	-.0065 mgm/dl/drck/day	.043
				High	.0054 mgm/dl/drck/day	.147

Alcohol use is associated with increasing GGPT levels among enlisted flying personnel, but the increase in GGPT falls smoothly with increasing exposure levels. On the other hand, alcohol use is associated with decreasing alkaline phosphatase levels among Ranch Hand officers in the low exposure group.

There are 2 interactions between exposure group and antibody to Hepatitis B antigen. Direct bilirubin levels are higher in enlisted flying personnel who are antibody positive and are in the low or high exposure groups. Direct bilirubin levels are lower in individuals who are antibody positive but in the medium exposure group. LDH is higher among enlisted ground Ranch Handers who are antibody positive and are in the low herbicide exposure group while LDH levels are lower among antibody positive individuals in the medium and high exposure groups.

An exposure-by-alcohol use interaction effect on cholesterol levels shows positive slopes in the low and high exposure categories but a negative slope in the medium exposure category.

Thus, of the 6 statistically significant interactions noted in this exposure index analysis only 1, the SGOT-degreasing chemical interaction, supports an interpretation of herbicide effect. But this interpretation is markedly weakened by the presence of the 5 uninterpretable patterns.

### 3. Urinalysis Determinations Related to Porphyrin Metabolism

Three components associated with porphyrin metabolism were determined and are analyzed here: uroporphyrin, coproporphyrin and d-aminolevulinic acid. Data addressing these 3 variables were analyzed looking for differences between the Ranch Hand and comparison groups and looking for associations with indexed herbicide exposure within the Ranch Hand group.

In examining the uroporphyrin, coproporphyrin and d-aminolevulinic acid data for Ranch Hand - comparison group differences, adjustments were accomplished for the following 6 variables: current alcohol use in drinks per day (ALC), blood urinary nitrogen (BUN), creatinine clearance (CCL), days of exposure to industrial chemicals (IC), days of exposure to degreasing chemicals (DC) and presence or absence of antibody to hepatitis B antigen. Adjustments were accomplished treating the dependent variable and all independent variables except antibody to hepatitis B antigen as continuous variables in a generalized linear model analysis. Since the compounds uroporphyrin, coproporphyrin and d-aminolevulinic acid are all measured in 24-hour urine collections, only data from subjects who complied with the full collection of urine are used in the analysis (620 Ranch Handers and 439 comparisons). Also, febrile participants and individuals with HB<sub>s</sub>Ag have been removed. In the adjusted analyses the dependent variable was normalized by using a logarithmic (base 10) transformation.

Table XIV-8 provides uroporphyrin, coproporphyrin and d-aminolevulinic acid unadjusted means, adjusted means and percent abnormality. For uroporphyrin, values greater than 60 were considered abnormal, for coproporphyrin, values greater than 235 and for d-aminolevulinic acid, values greater than 7000 were counted as abnormal.

Table XIV-8

UNADJUSTED MEANS, ADJUSTED MEANS AND PERCENT ABNORMALITY  
FOR THREE COMPOUNDS RELATED TO PORPHYRIN METABOLISM

		Unadjusted Means	Adjusted Means	% Abnormal
Uroporphyrin	RH	30.5	*	6.5%
	COM	30.8	*	6.8%
Coproporphyrin	RH	31.2	*	0.2%
	COM	30.8	*	0.0%
d-aminolevulinic acid	RH	2328.9	2337.1	0.0%
	COM	2383.2	2371.4	0.0%

\* adjusted means not represented due to interaction

Table XIV-9

SUMMARY OF RESULTS UNMATCHED ANALYSES  
OF THREE COMPOUNDS RELATED TO PORPHYRIN METABOLISM  
P-VALUES FOR MODELS WITH INTERACTION

VAR	Gp	ALC	BUN	CCL	IC	DC	Anti HBsAg	Gp x ALC	Gp x BUN	Gp x CCL	Gp x IC	Gp x DC	Gp x Anti HBsAg
URO	.227	-	<.001	<.001	-	-	-	-	.077	-	-	-	-
COPRO	.490	-	<.001	<.001	-	.049	-	.045	.097	-	-	-	-
ALA	.145	-	-	<.001	-	-	.014	-	-	-	-	-	-

Table XIV-9 displays the detailed analyses. No overall group differences are observed. With uroporphyrin a borderline significant group-by-BUN interaction ( $P = 0.077$ ) was observed. In the Ranch Hand group, the uroporphyrin-BUN slope was  $-0.010$  uroporphyrin logarithm units per BUN unit, while the comparison slope was steeper ( $-0.017$ ). A borderline group-by-BUN interaction was also noted in the coproporphyrin data. In the Ranch Hand group, the coproporphyrin-BUN slope was  $-0.014$  coproporphyrin logarithmic units per BUN unit, while the comparison slope was again steeper ( $-0.023$ ). Lastly, a group-by-alcohol interaction was detected in the coproporphyrin data ( $P = 0.045$ ). The Ranch Hand slope was positive ( $+0.013$ ) while the comparison slope was negative ( $-0.008$ ).

Table XIV-10

SUMMARY OF P VALUES FOR EXPOSURE INDEX ANALYSES OF THREE COMPOUNDS  
RELATED TO PORPHYRIN METABOLISM

<u>VAR</u>	<u>OCC</u> <u>CAT</u>	<u>EXP</u> <u>CAT</u>	<u>ALC</u>	<u>BUN</u>	<u>CCL</u>	<u>IC</u>	<u>DC</u>	<u>aHb</u>	<u>EXP</u> <u>x</u> <u>ALC</u>	<u>EXP</u> <u>x</u> <u>BUN</u>	<u>EXP</u> <u>x</u> <u>CCL</u>	<u>EXP</u> <u>x</u> <u>IC</u>	<u>EXP</u> <u>x</u> <u>DC</u>	<u>Exp x</u> <u>Anti</u> <u>HB<sub>s</sub>Aq</u>
URO	OFF	.207	-	-	<.001	-	-	-	-	-	-	-	.033	-
	ENL F.	.670	-	-	-	-	-	-	-	-	-	-	-	-
	ENL G.	.882	-	.010	.050	-	-	-	-	-	-	-	-	-
COPRO	OFF	.630	-	-	.022	.035	-	-	-	-	-	-	-	-
	ENL F.	.498	-	<.001	-	-	-	-	-	-	-	-	-	-
	ENL G.	.699	-	.016	.015	-	-	-	-	-	-	.016	-	-
ALA	OFF.	.279	-	-	<.001	-	-	-	-	-	-	-	-	-
	ENL F.	.135	-	-	<.001	-	-	-	.028	-	-	-	-	-
	ENL G.	.312	-	-	<.001	.020	-	-	-	-	-	.040	.042	-

Table XIV-11

TABLE OF UNADJUSTED MEANS FOR THREE COMPOUNDS  
RELATED TO PORPHYRIN METABOLISM

<u>Variable</u>	<u>Occupational</u> <u>Category</u>	<u>N</u>	<u>Low</u> <u>Exposure</u>	<u>Medium</u> <u>Exposure</u>	<u>High</u> <u>Exposure</u>
Uroporphyrin	Officers	212	28.9	26.9	31.3
	Enlisted Fly.	106	38.7	27.8	31.6
	Enlisted Gnd.	282	31.1	32.4	29.8
Coproporphyrin	Officers	212	32.4	26.7	29.9
	Enlisted Fly.	106	36.4	31.1	32.5
	Enlisted Gnd.	282	31.6	30.9	32.8
d-amino levulinic Acid	Officers	212	2221	2312	2211
	Enlisted Fly.	106	2460	2510	2381
	Enlisted Gnd.	282	2290	2441	2271

Table XIV-12

EXPOSURE-COVARIATE INTERACTIONS FOR THREE COMPOUNDS  
RELATED TO PORPHYRIN METABOLISM

<u>Variable</u>	<u>Occupational Category</u>	<u>Interaction</u>	<u>P Value for Interaction</u>	<u>Exposure Level</u>	<u>Slope</u>
Uroporphyrin	Officer	Exp x DC	.033	Low	-.000043
				Med	.000074
				High	.000190
Copro- porphyrin	Enlisted Ground	Exp x IC	.016	Low	.301 X 10 <sup>-4</sup>
				Med	-.540 X 10 <sup>-4</sup>
				High	.176 X 10 <sup>-4</sup>
d-amino levulinic acid	Enlisted Flying	Exp x ALC	.028	Low	.00045
				Med	-.02922
				High	.01445
d-amino levulinic acid	Enlisted Ground	Exp x IC	.040	Low	-.1450 X 10 <sup>-4</sup>
				Med	-.2944 X 10 <sup>-4</sup>
				High	.0315 X 10 <sup>-4</sup>
d-amino levulinic acid	Enlisted Ground	Exp x DC	.042	Low	-.0538 X 10 <sup>-4</sup>
				Med	.0398 X 10 <sup>-4</sup>
				High	.0394 X 10 <sup>-4</sup>

The literature indicates elevated porphyrin compound excretion resulting from sufficient dioxin exposure. The pattern found here is one of higher Ranch Hand uroporphyrin or coproporphyrin levels relative to comparisons when there are concomitantly higher BUN levels, or, in the case of coproporphyrin, when there is higher alcohol ingestion. No overall group differences are observed.

Tables XIV-10, XIV-11 and XIV-12 display the results of exposure index analyses within the Ranch Hand group. Starting with Table XIV-10, no statistically significant overall group differences are seen and 5 statistically significant ( $P \leq 0.050$ ) group-covariate interactions are noted. Table XIV-11 displays unadjusted group means for the porphyrin metabolism related variables and, as indicated by the statistical testing of overall group differences, no trends with exposure index are observed.

The 5 exposure-by-covariate interactions are listed in Table XIV-12; however, only the exposure index by degreasing chemical interactions follow a classical dose-response pattern. Specifically, Ranch Hand officers with greater herbicide exposure, as measured by the exposure index, have greater increases in uroporphyrin output in response to degreasing chemical exposures than do Ranch Hand officers with less herbicide exposure. The same pattern is seen in the enlisted ground d-aminolevulinic acid data.

#### 4. Clinical Variables

Sixteen of 1027 Ranch Handers (1.56%) were diagnosed as having hepatomegaly while 6 of 769 comparisons (0.78%) had that finding ( $P = 0.138$ ) with an approximate 70% power. In the Ranch Hand group, the cases of hepatomegaly appear to be randomly distributed within the 3 exposure categories; however, due to the small number of cases statistical testing is not powerful. These data on hepatomegaly are shown in Table XIV-13 (febrile participants and individuals with HBsAg have been removed).

Table XIV-13

CASES OF HEPATOMEGALY IN THE RANCH HAND COHORT BY  
OCCUPATION AND EXPOSURE CATEGORY

<u>Occupational Category</u>	<u>Exposure Index</u>					
	<u>Low</u>		<u>Medium</u>		<u>High</u>	
	<u>Cases</u>	<u>N</u>	<u>Cases</u>	<u>N</u>	<u>Cases</u>	<u>N</u>
Officers	2	110	2	124	2	123
Enlisted Flying	1	59	2	58	2	63
Enlisted Ground	0	148	3	176	1	147

Eighteen of 1027 Ranch Handers (1.75%) reported an enlarged liver during response to questionnaire inquiry while 13 of 760 comparisons (1.71%) reported the same.

The study questionnaire also inquired about a medical history of hepatitis, jaundice, cirrhosis, and a general category called other liver conditions. Ranch Hand and comparison responses to these questions are shown in Table XIV-14. Ranch Hand respondents differ from comparisons only in the other liver category. Thirteen of the 16 Ranch Handlers reporting other liver conditions have had their report verified by medical record. One comparison has had his condition verified. A display of the verified findings is shown in Table XIV-15 (febrile individuals and HBsAg positive individuals were left in the analysis).

Table XIV-14

SPECIFIC LIVER DISORDERS REPORTED ON QUESTIONNAIRE

<u>Reported Event</u>	<u>Ranch Hand</u>		<u>Comparison</u>		<u>P Value</u>
	<u>Yes</u>	<u>No</u>	<u>Yes</u>	<u>No</u>	
Hepatitis	40	1005	32	741	>0.50
Jaundice	44	1001	35	738	>0.50
Cirrhosis	4	1041	3	770	>0.50
Other	16	1029	2	771	0.004

Table XIV-15

OTHER LIVER CONDITIONS REPORTED BY  
STUDY PARTICIPANTS AND VERIFIED BY MEDICAL RECORDS

<u>Ranch Hand:</u>	<u>ICD Code</u>	<u>Code Meaning</u>	<u>Number</u>
	2724	Hyperlipidemia	1
	570	Liver necrosis	1
	5739	Unspecified	10
	7904	Enzyme elevation	1
Comparison:	5719	Chronic unspecified	1

Table XIV-16

REPORTED SKIN PATCHES, BRUISES OR SENSITIVITY  
IN RANCH HAND PARTICIPANTS BY  
OCCUPATION AND EXPOSURE CATEGORY

<u>Occupational Category</u>	<u>Exposure Index</u>								
	<u>Low</u>			<u>Medium</u>			<u>High</u>		
	<u>Cases</u>	<u>%</u>	<u>N</u>	<u>Cases</u>	<u>%</u>	<u>N</u>	<u>Cases</u>	<u>%</u>	<u>N</u>
Officers	36	32.4	111	48	37.5	128	44	35.2	125
Enlisted Flying	27	45.8	59	28	47.5	59	37	56.1	66
Enlisted Ground	74	49.0	151	82	45.8	179	76	51.4	148

Seeking historical evidence of porphyric symptoms, questions concerning skin changes that could have been associated with porphyria cutanea tarda were asked (specifically, skin patches, bruisability or sensitivity). Of 1045 Ranch Hand respondents, 462 or 44.2% reported these skin symptoms while 278 of 773 comparisons or 36.0% reported these conditions. These reported cases indicate a statistically significant group difference ( $P < 0.001$ ); however, no regression with exposure index was noted (data given in Table XIV-16).

The historical and hepatomegaly data support an interpretation of some group difference. However, no positive association with herbicide exposure has been noted.

##### 5. Summary and Conclusion

Ranch Handlers have slightly greater GGPT and LDH levels than the comparisons while having lower cholesterol levels. Also, Ranch Hand SGOT, SGPT and LDH levels are more highly correlated to (and therefore may be more influenced by) materials with an hepatic effect, namely, alcohol, degreasing compounds and industrial chemicals. No group differences were noted in alkaline phosphatase or bilirubin levels.

Borderline statistically significant group differences have been detected in uroporphyrin and coproporphyrin levels in association with BUN, and in coproporphyrin levels in association with alcohol ingestion. No overall group differences were detected in these compounds or delta aminolevulinic acid values.

Twice as many Ranch Handlers as comparisons had enlarged livers on physical examination, but this difference was not statistically significant. Statistically significant group differences were noted in the occurrence of miscellaneous liver disorders exclusive of hepatitis, jaundice and cirrhosis, verified by



medical record review. Ranch Handers self reported 23% more skin changes of the type associated with porphyria cutanea tarda than did the comparison participants, and the group difference was statistically significant. Clinically apparent porphyria was not evident at physical examination.

The observed group differences in liver-related biochemical variables found in the blood, and in porphyrin metabolism compounds found in the urine are most likely of minor or negligible medical importance at the present time. The verified reports of liver morbidity are of greater clinical interest.

The exposure index analyses do not support an interpretation of herbicide effect with respect to any of the group differences summarized.